Transcriptional Regulation of the Human Manganese Superoxide Dismutase (MnSOD) Gene

Sureerut Porntadavity & Daret K. St. Clair


Sureerut Porntadavity

Graduate Center for Toxicology,
University of Kentucky
361 Health Sciences Research Building
Lexingtion, KY 40536, U.S.A.


Daret K. St. Clair

dstcl00@pop.uky.edu, Tel.: (859) 257-3720; Fax: (859) 323-1059

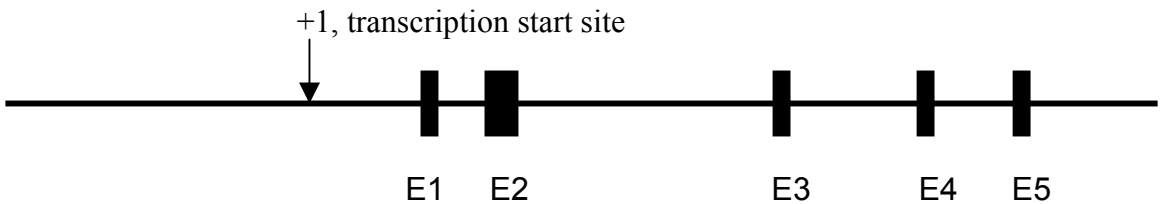
Why regulation of MnSOD is important?

- MnSOD is essential for the survival of aerobic life
- increased MnSOD level protects normal tissues against oxidative stress-mediated pathological conditions
- MnSOD suppresses neoplastic transformation, cancer phenotypes, and metastatic potential
- MnSOD alters gene expression associated with proliferation/apoptosis

(Carlioz A. and Touati D., 1986; Wong G.H. *et al*., 1989; St. Clair D.K. *et al*., 1992; Church S.L. *et al*., 1993; St. Clair D.K. *et al*., 1994; *Safford S.E. et al*., 1994; Li Y. *et al.,* 1995; Kiningham K.K. *et al*., 1997; Drane P. *et al*., 2001; Zhao Y. *et al*., 2002)

The human MnSOD gene (GenBank Accession no.S77127)

- locates on chromosome 6q25
- is a single copy gene
- consists of 5 exons and 4 introns

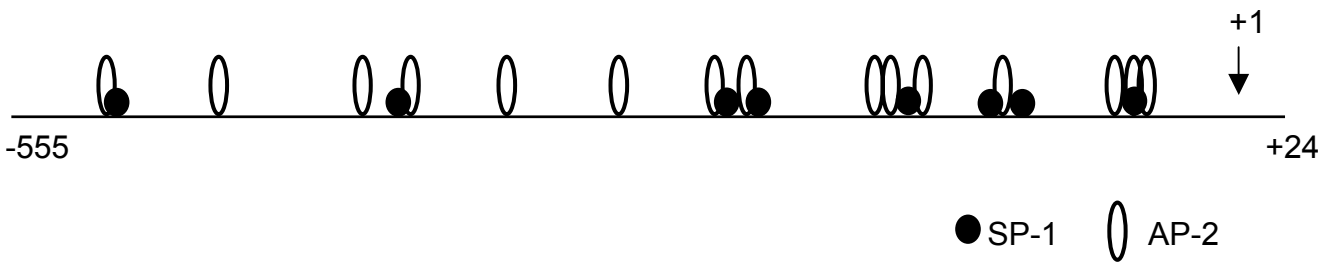


- is transcribed and processed into multiple transcripts (1, 4, and 6 kb)
- is encoded for a 222-amino acid protein containing a 24 amino acids leader peptide necessary for mitochondrial targeting

(Beck Y. *et al.*, 1988; Ho Y-S. and Crapo J.D., 1988; Wispe J.R. *et al.*, 1989; Church, S.L., 1990; Church S.L. *et al.*, 1992; Wan X.S. *et al.*, 1994)

The human MnSOD promoter

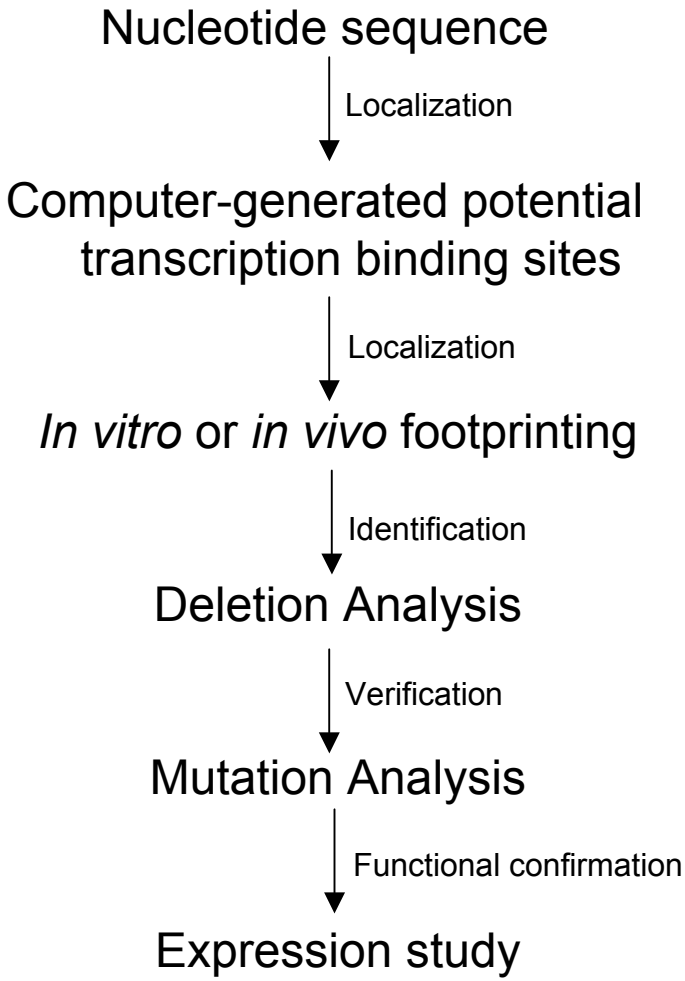
- consists of 78% GC without a TATA or CAAT box
- contains multiple SP-1 and AP-2 binding sites



- may also contain binding site for other transcription factors especially zinc finger family members such as Sp3, AML-1a, MZF1

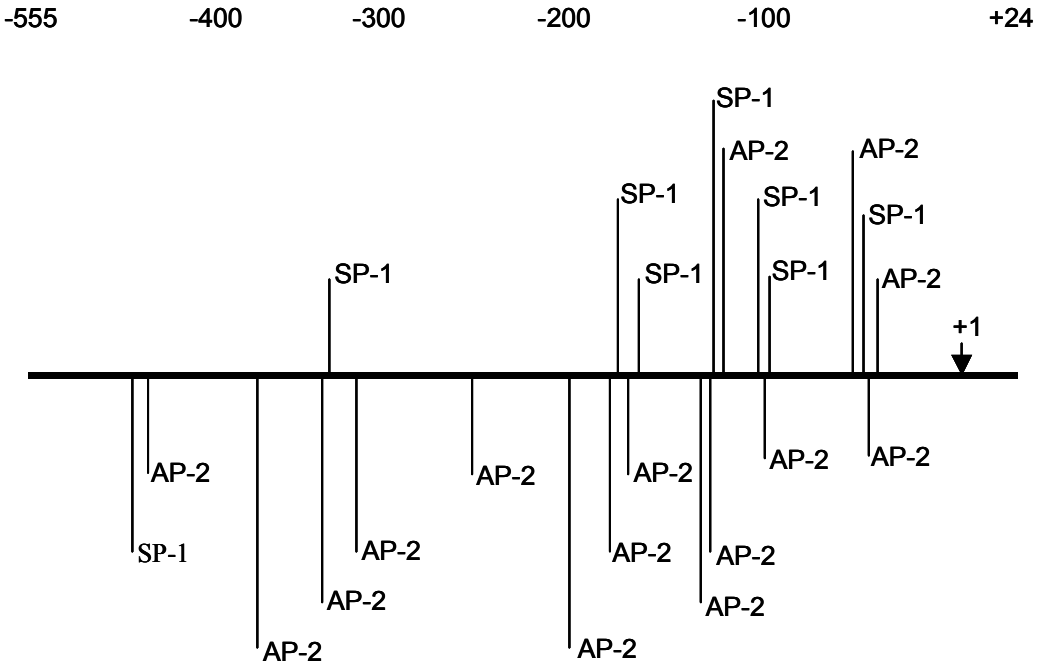
(Wan X.S. *et al.*, 1994; Yeh C-C. *et al.*, 1998)

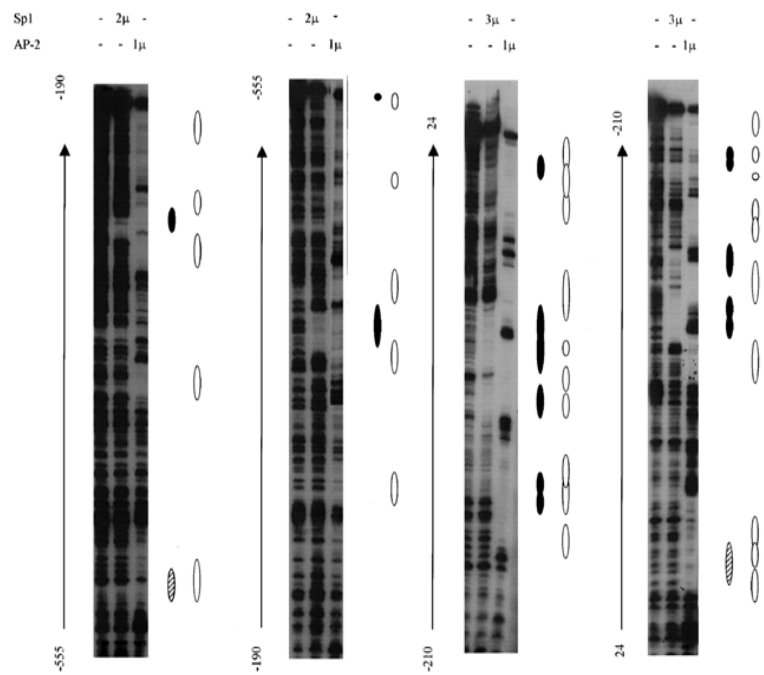
Methods for identification of regulatory elements



Localization:

MnSOD promoter contains overlapping SP-1 and AP-2 binding sites





Computer Analysis Computer analysis depicted the potential binding sites for SP-1 and AP-2 on the MnSOD promoter region (marked on the top). *In vitro* footprinting (right) revealed additional SP-1 and AP-2 binding sites that were not identified by computer analysis (marked on the bottom).

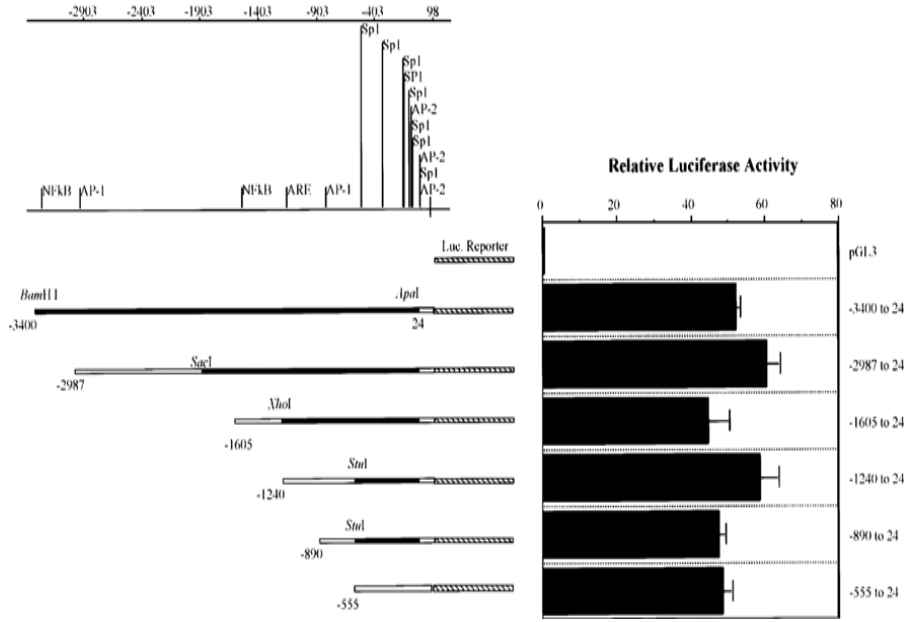
Note: -These binding sites may not function *in vivo*.
- Some functional binding sites may not be identified by computer analysis.

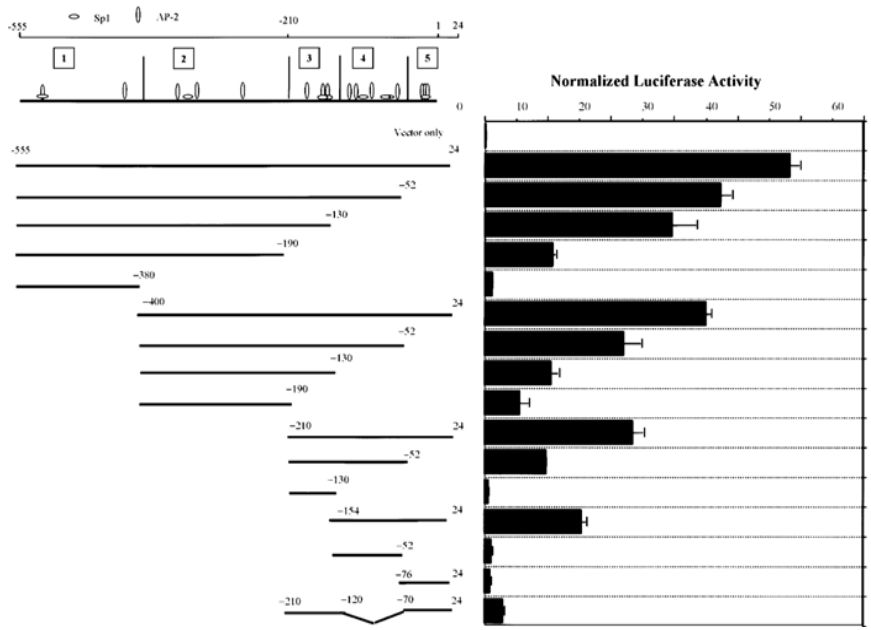
***In vitro* footprinting.** SP-1 and AP-2 binding sites were identified in both strand of the promoter region by DNase I digestion with purified SP-1 or AP-2 protein. The binding site for SP-1 (solid oval) and AP-2 (open oval) are shown. The shaded oval is the binding site for SP-1 that can not be detected, but can be detected on the complementary strand.

(Wan X.S. *et al.*, 1994; Xu Y. *et al.*, 2002)

Identification:

The 5’ flanking region from -555 of the transcription start site is sufficient for a high-level transcription





Deletion Analysis. Fragments containing various length of the 5’ flanking region were subcloned into pGL3 to drive the expression of the reporter gene. The promoter acitivity of each fragment was determined by luciferase activity assay.

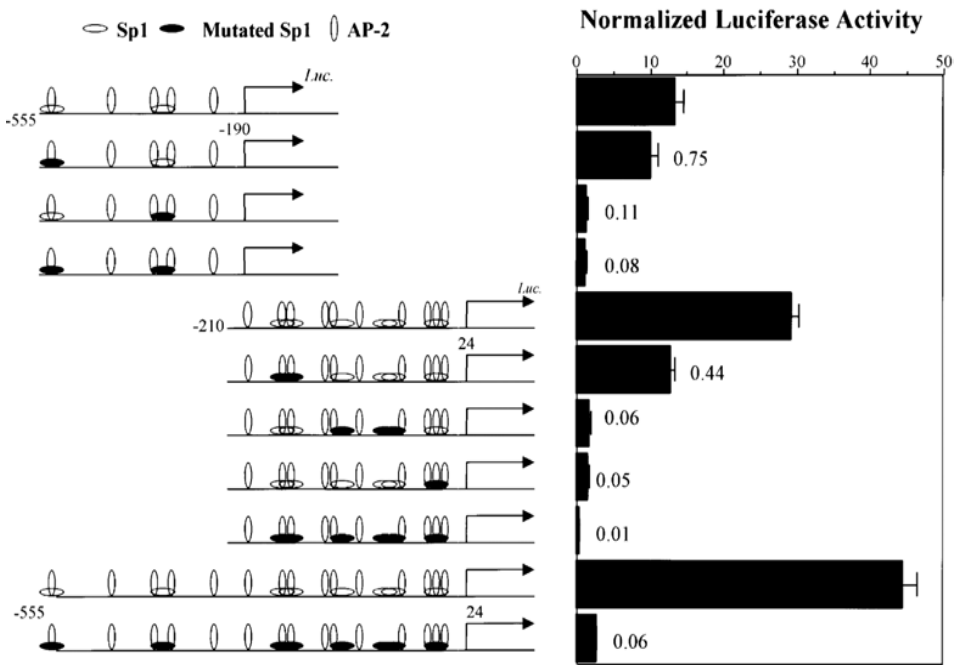
(Xu Y. *et al.*, 2002)

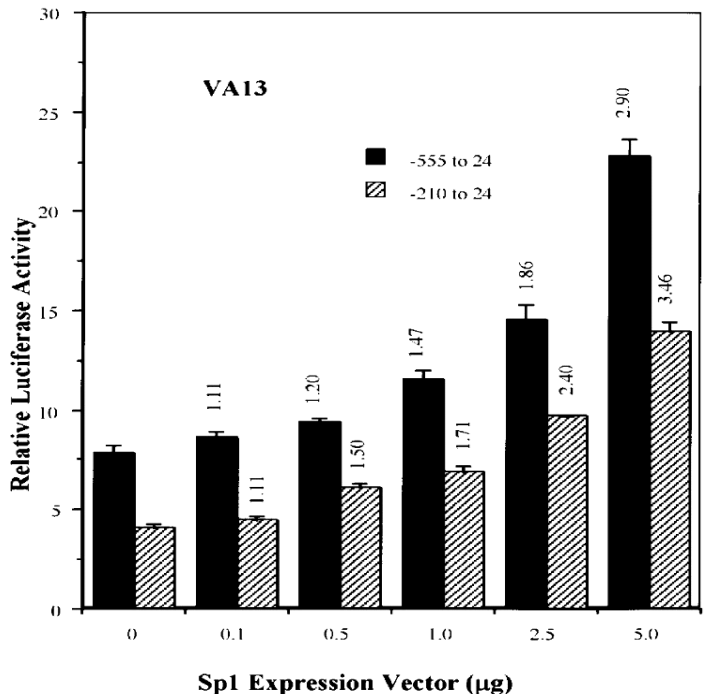
Deletion analysis. Deletion of five binding domains containing 8 SP-1 binding sites and 15 AP-2 binding sites in the five motifs are illustrated at the top. Deletion fragments were generated by PCR based on the binding motifs and then subcloned into pGL3 to drive the expression of the reporter gene.

Note: - At least two binding motifs are needed for a minimal transcription.

Verification and Functional confirmation:

SP-1 plays a positive role in the constitutive transcription





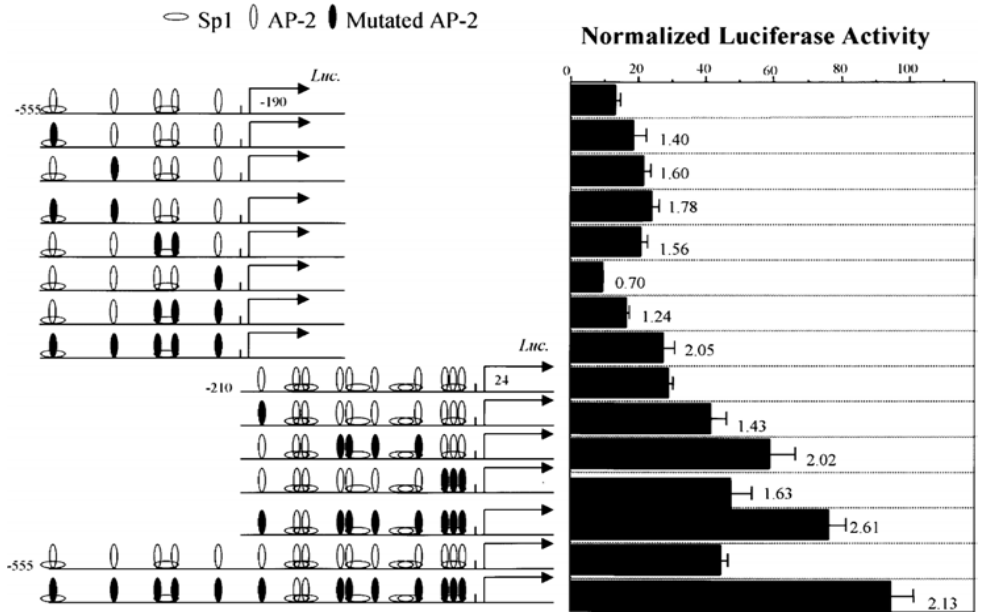
Mutation Analysis. SP-1 binding sites were mutated by site-directed mutagenesis and then subcloned into pGL3 to drive the expression of the reporter gene. The role of each SP-1 binding site was determined by luciferase activity assay. The number by the error bars indicated the fold reduction from non-mutated control.

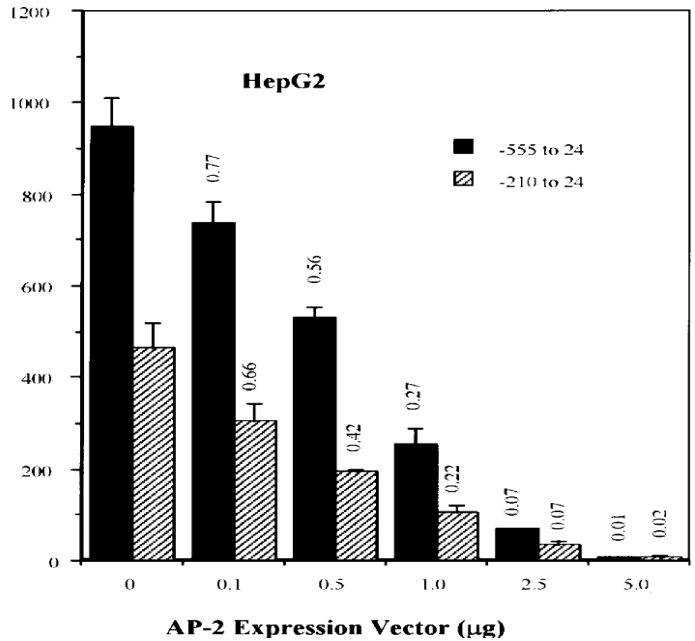
Expression study. The effect of SP-1 on the MnSOD promoter (-555 to +24 and -210 to +24). VA-13 cells were co-transfected with the promoter and various amounts of the SP-1 expression vector. The fold induction is indicated by the number above the error bars.

(Xu Y. *et al.*, 2002)

Verification and Functional confirmation:

AP-2 plays a negative role in the constitutive transcription





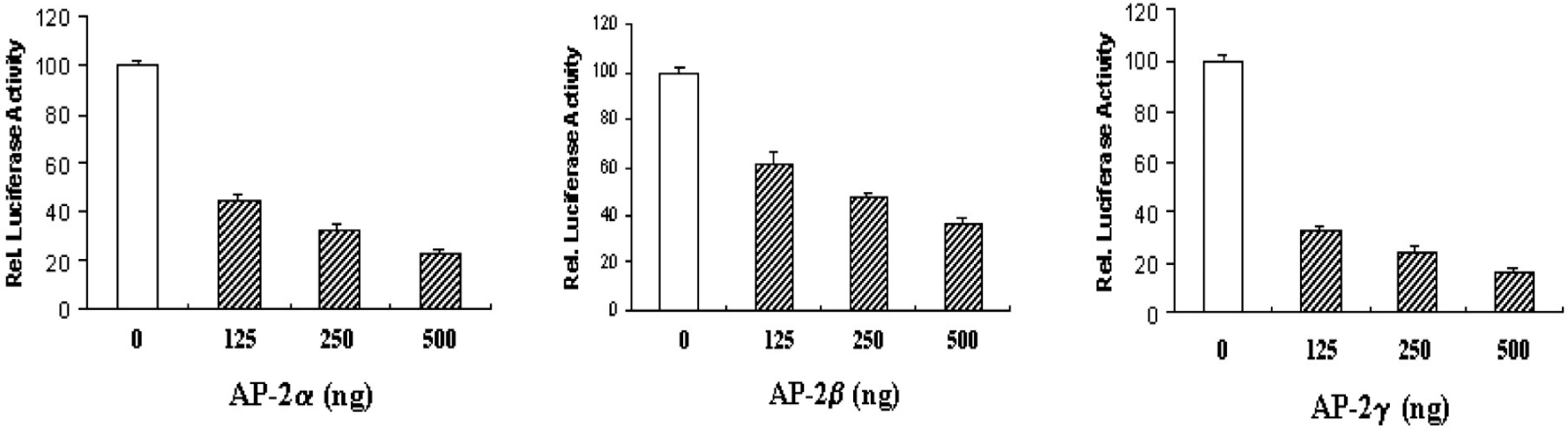
Mutation analysis. AP-2 binding sites were mutated by site-directed mutagenesis and then subcloned into pGL3 to drive the expression of the reporter gene. The role of each AP-2 binding site was determined by luciferase activity assay. The number by the error bars indicated the fold induction from non-mutated control.

Expression study. The effect of AP-2 on the human MnSOD promoter (-555 to +24 and -210 to +24). HepG2 cells were co-transfected with the promoter and various amounts of the AP-2 expression vector. The fold reduction is indicated by the number above the error bars.

(Xu Y. *et al.*, 2002)

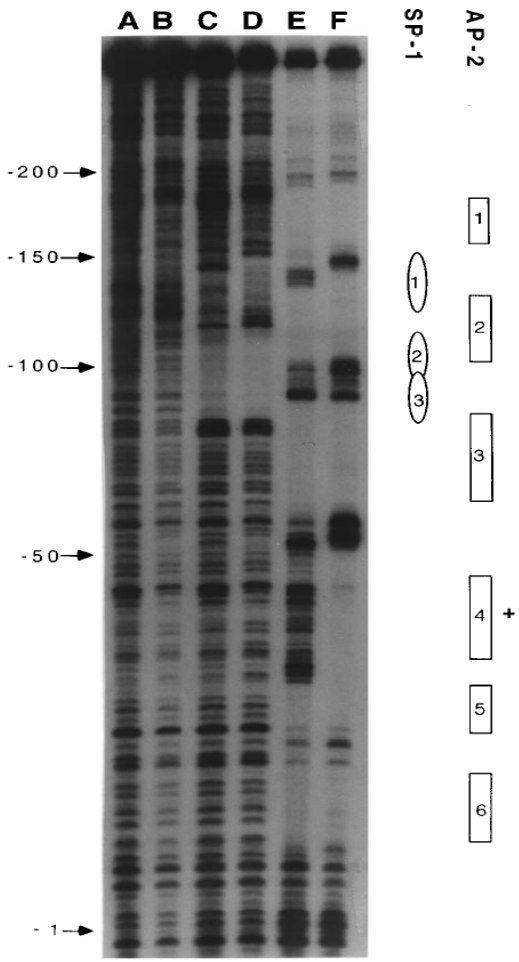
Functional confirmation:

A family of AP-2 proteins represses promoter activity



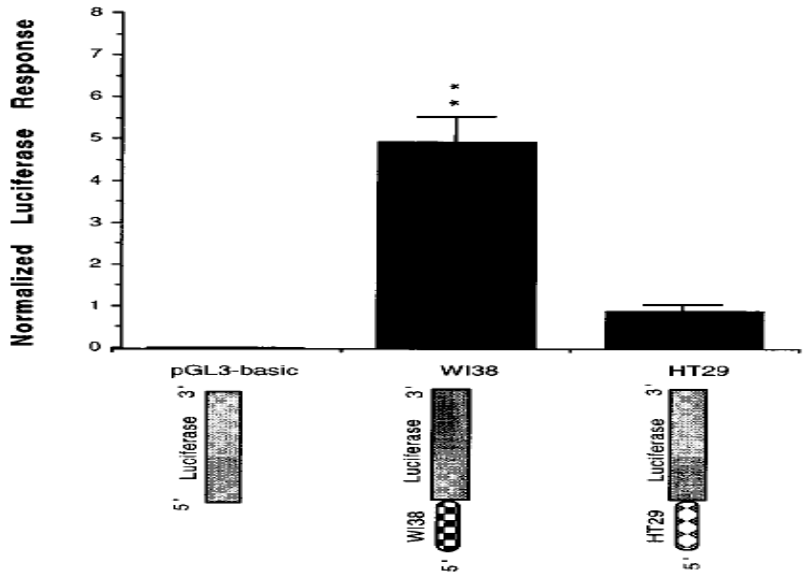
Expression study. HepG2 cells were co-transfected with MnSOD promoter (-555/+24) and three different isoforms of AP-2. The effect of each AP-2 isoform was determined by luciferase activity assay. (From Zhu C-H. *et al*., *JBC*. 276, 2001. With permission)

Mutations in the promoter may contribute to decreased expression of the human MnSOD gene in some cancer cells



Footprinting analysis. Dnase I footprinting analysis of the human MnSOD promoter using purified SP-1 and AP-2 proteins. Normal promoter without protein (A), with SP-1 protein (C) with AP-2 protein (E); Mutated promoter without protein (B), with SP-1 protein (D), with AP-2 protein (F). The binding patterns of SP-1 and AP-2 are indicated on the right. (+) represents an additional AP-2 binding site in the mutated promoter.

Effect of mutations in the promoter on gene expression. Human fibroblast cells were transfected with pGL3 plasmids containing promoter from WI38 (normal) or mutated promoter (HT-29). The promoter activity was measured by luciferase activity assay.



(Xu Y. *et al.*, 1999)

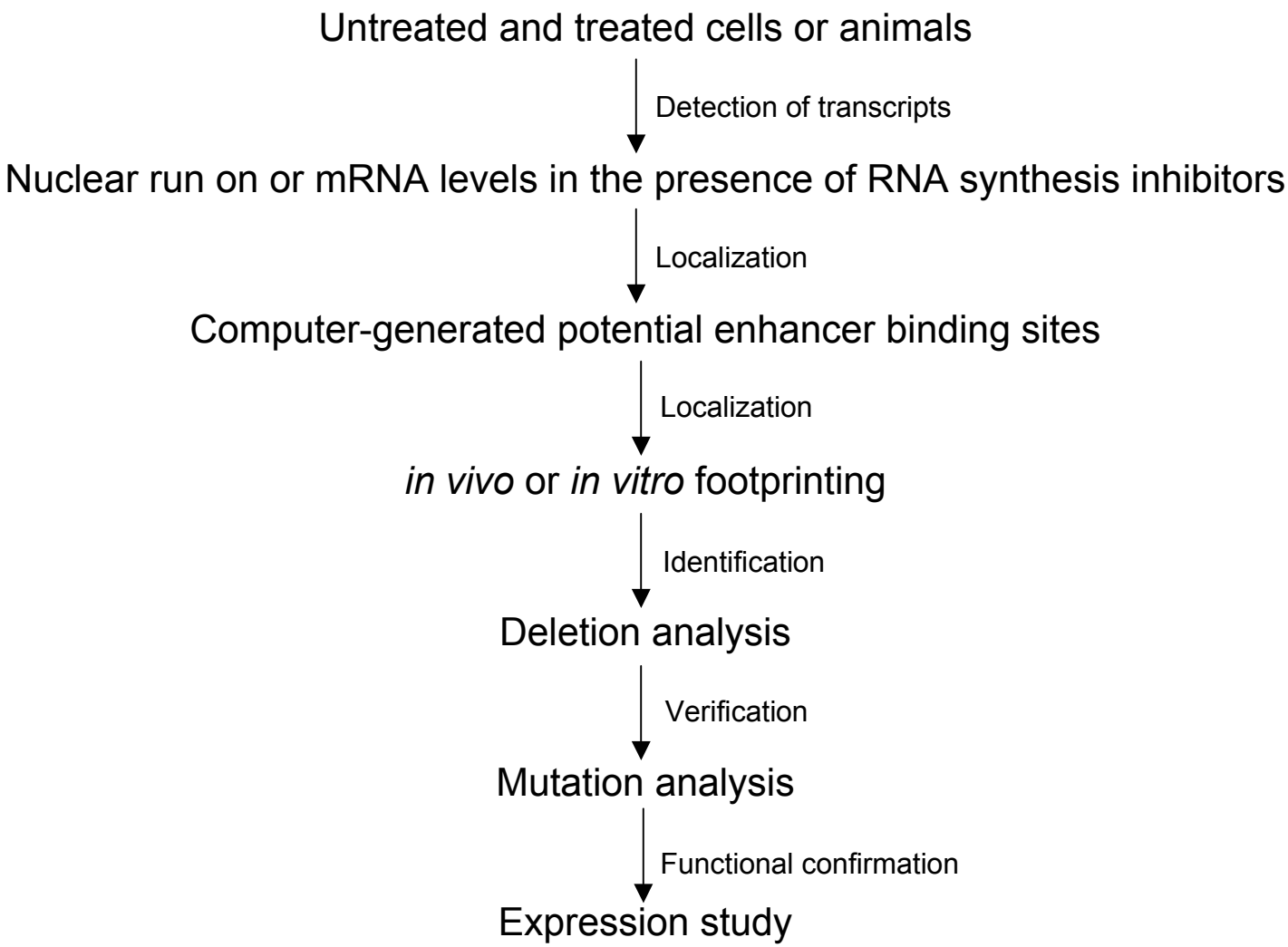
MnSOD is inducible

- MnSOD gene is highly inducible by various agents and conditions that cause oxidative stress. Enhancer elements have been identified in the 5' flanking region as well as in an intronic region of the human MnSOD gene
- Enhancer elements identified in the 5' flanking region including MnSOD TPA-responsive element (MSTRE) located between -1292 and -1202 on the 5' flanking region of the human MnSOD gene in lung carcinoma cells, and SP-1 mediated TPA-responsive elements in the GC rich region of the promoter in hepatocarcinoma cells
- Enhancer elements identified in the intronic region is located between 1741 and 2083. It contains binding sites for several transcription factors including C/EBP-1, -2, -X, NF- κB, and NF-1

Note: The elements identified in the 5' flanking region appear to function in cell type specific manners.

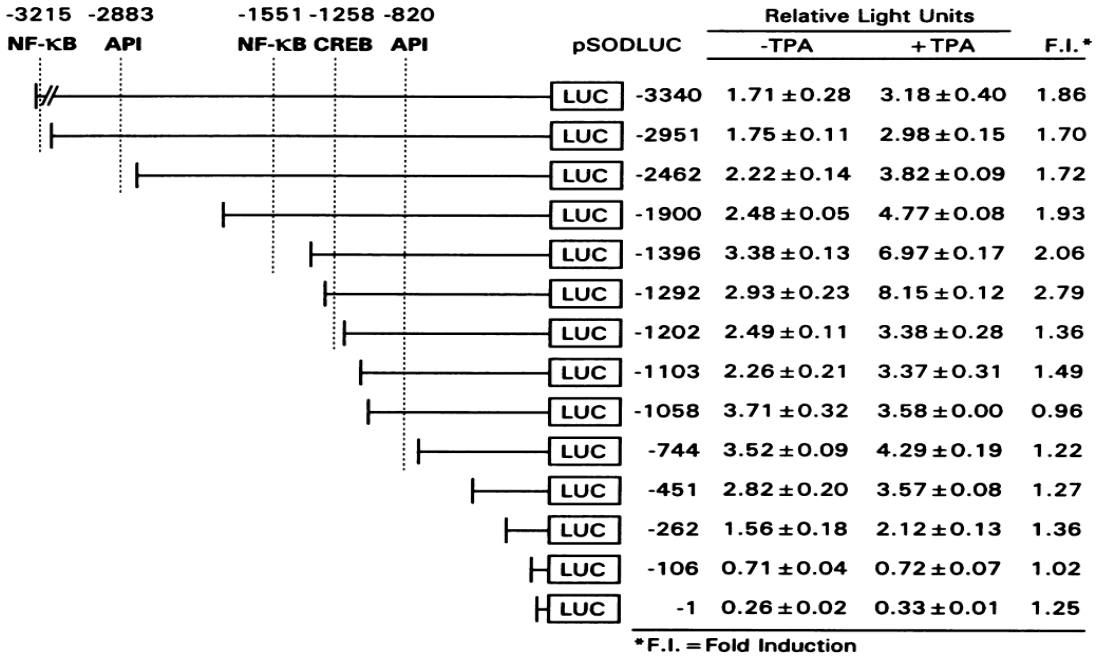
(Wong G.H. and Goeddel D.V., 1988; Wong G.H. *et al.*, 1989; Visner G.A. *et al.*, 1990; Fujii J. and Taniguchi N., 1991; Xu Y. *et al.*, 1999; Kim H-P. *et al.*, 1999; Porntadavity S. *et al.*, 2001)

Methods for Identification inducible elements



Identification:

TPA-responsive element (MSTRE) located between -1292 and -1202 on the 5' flanking region of the human MnSOD gene



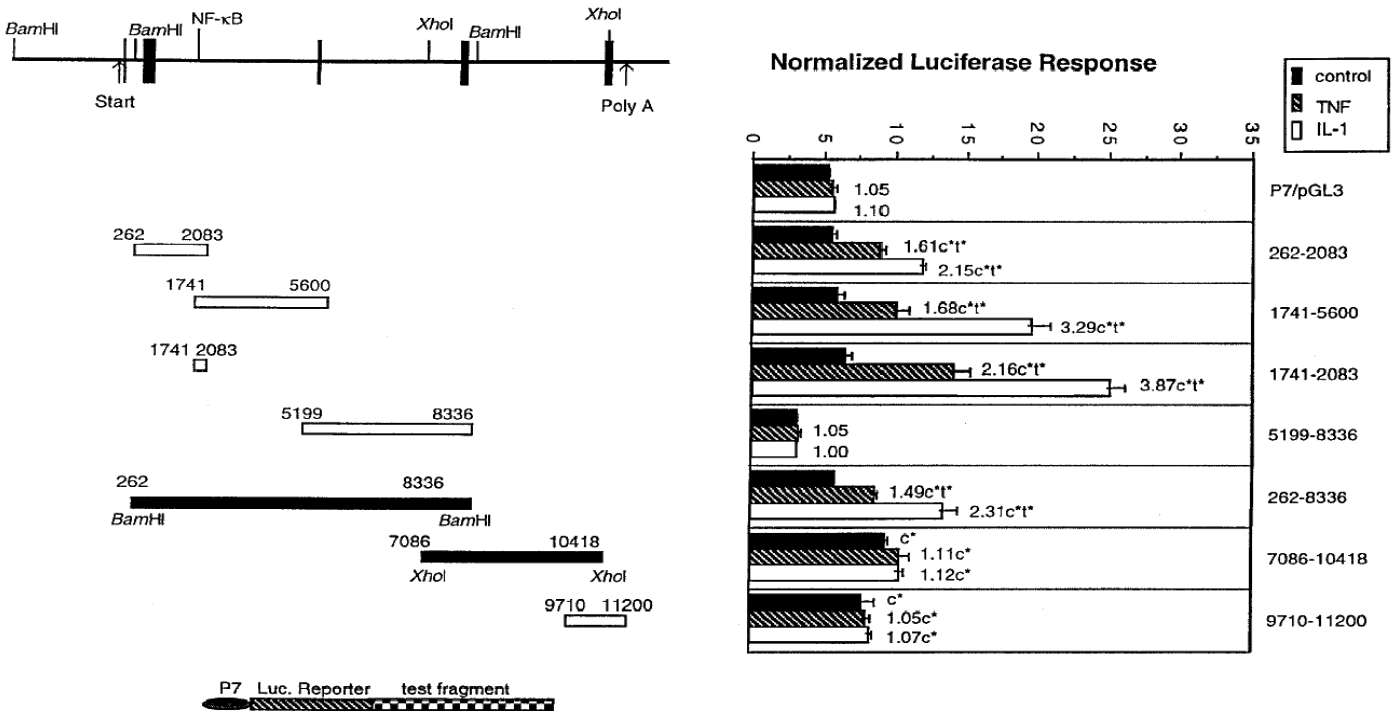
pSODLUC	Relative Light Units -TPA	Relative Light Units +TPA	F.I.*
-3340	1.71 ± 0.28	3.18 ± 0.40	1.86
-2951	1.75 ± 0.11	2.98 ± 0.15	1.70
-2462	2.22 ± 0.14	3.82 ± 0.09	1.72
-1900	2.48 ± 0.05	4.77 ± 0.08	1.93
-1396	3.38 ± 0.13	6.97 ± 0.17	2.06
-1292	2.93 ± 0.23	8.15 ± 0.12	2.79
-1202	2.49 ± 0.11	3.38 ± 0.28	1.36
-1103	2.26 ± 0.21	3.37 ± 0.31	1.49
-1058	3.71 ± 0.32	3.58 ± 0.00	0.96
-744	3.52 ± 0.09	4.29 ± 0.19	1.22
-451	2.82 ± 0.20	3.57 ± 0.08	1.27
-262	1.56 ± 0.18	2.12 ± 0.13	1.36
-106	0.71 ± 0.04	0.72 ± 0.07	1.02
-1	0.26 ± 0.02	0.33 ± 0.01	1.25

*F.I. = Fold Induction

Deletion analysis. Fragments of 5' flanking region were subcloned into pGL3 to drive the expression of the reporter gene. The promoter activity of each fragment was measured by luciferase activity assay. The fold induction is indicated by the number in the last column.
(From Kim H-P. *et al.*, JBC 274, 1999. With permission.)

Identification:

A region between 1741 and 2083 in intron 2 is sufficient for the induction of the human MnSOD gene by TNF and IL-1.

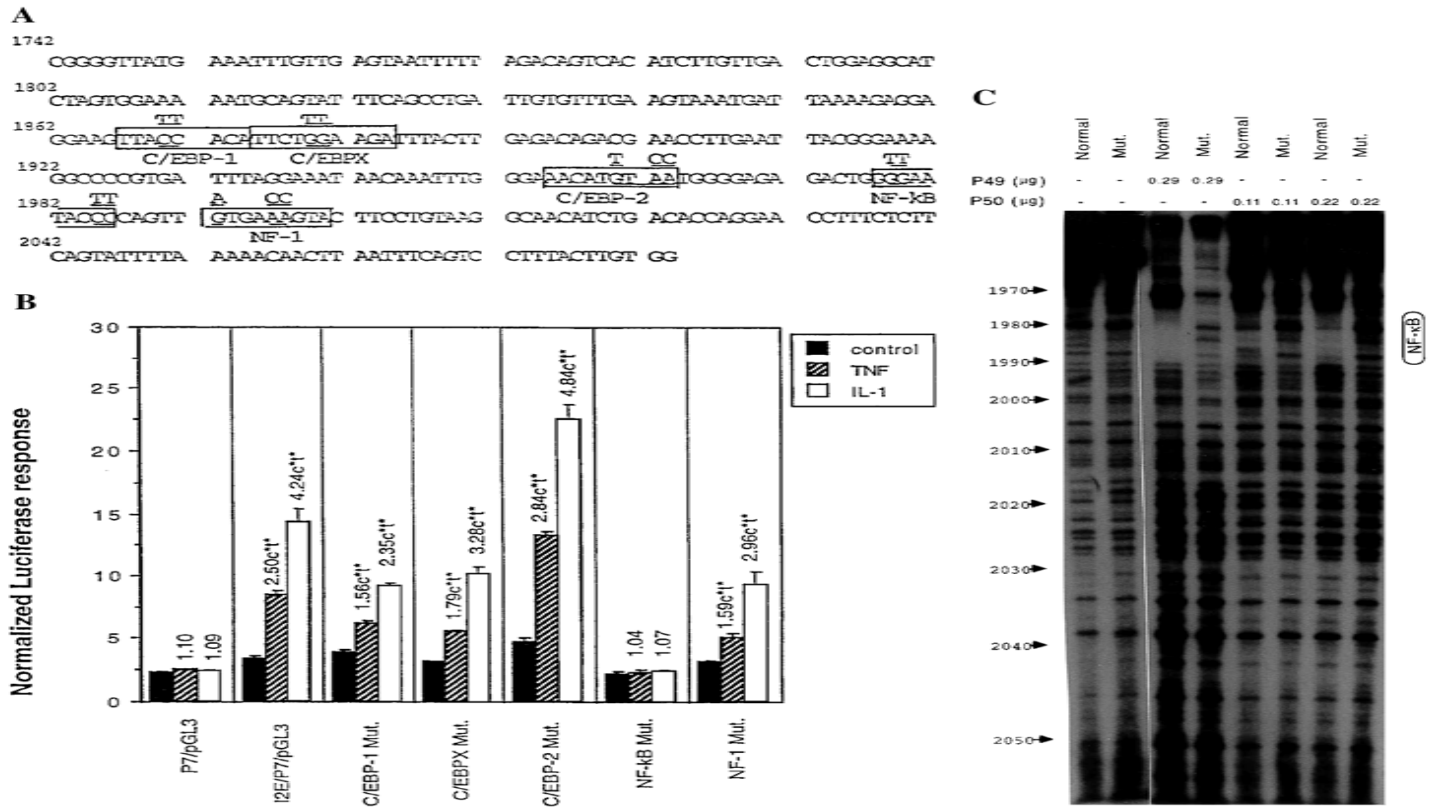


Deletion analysis. Fragments of the MnSOD gene were generated by restriction digestion (solid box) or PCR (open box) and then subcloned downstream of the reporter gene (pGL3) driven by the promoter (P7). The enhancer capability of each fragment was determined by luciferase activity assay. The number by the error bars indicate the fold induction compare to untreated cells. t*, significant differences between treatment and nontreatment using the same construct ($p \leq 0.01$). c*, significant differences between the constructs and the vector control (P7/pGL3) receiving the same treatment ($p \leq 0.01$).

(Xu Y. *et al.*, 1999)

Verification:

An intronic enhancer contains NF-κB binding site responsible for MnSOD induction



Mutaion analysis. (A) The binding site for transcription factors in the intronic enhancer are boxed. The mutated bases change are on the top. (B)The role of each binding site was determined by luciferase activity assay. The number by the error bars indicate the fold induction. (C) Footprinting analysis of the NF-κB binding site in the I2E fragment using purified p49 or p50 proteins. The NF- κB binding site is marked on the right.

(Xu Y. *et al*., 1999)

Conclusions about the human MnSOD gene:

- is a single copy gene located on chromosome 6q25
- The promoter region contains multiple SP-1 and AP-2 binding sites
- SP-1 plays a positive role in the transcription of the gene
- AP-2 plays a negative role in the transcription of the gene
- is highly inducible by various agents and conditions
- Enhancer elements are located in the 5' flanking region as well as in an intronic region

References

- Beck Y., Oren R., Abramovich C., Amit B., Levanon A., Gorecki M., and Hartman R.J. (1988) Moleculare structure and expression of the human Mn-SOD gene. J. Cell Biochem Suppl. 12A, 37.

- Carlioz A., and Touati D. (1986) Isolation of superoxide dismutase mutants in *Escherichia coli*: Is superoxide dismutase necessary for aerobic life? EMBO J. 5, 623-630.

- Church S.L. (1990) Manganese superoxide dismutase: nucleotide and deduced amino acid sequence of a cDNA a new human transcript. Biochim. Biophys. Acta 1087, 250-252.

- Church S.L., Grant J.W., Meese E.U., and Trent J.M. (1992) Sublocalization of the gene encoding manganese superoxide dismutase (MnSOD/SOD2) to 6q25 by fluorescence *in situ* hybridization and somatic cell hybride mapping. Genomics. 14, 823-825.

- Church S.L., Grant J.W., Ridnour L.A., Oberley L.W., Swanson P.E., Meltzer P.S., and Trent J.M. (1993) Increased manganese superoxide dismutase expression suppress the malignant phenotype of human melanoma cells. Proc. Natl. Acad. Sci. USA 90, 3113-3117.

- Drane P., Bravard A., Bouvard V., and May E. (2001) Reciprocal down-regulation of p53 and SOD2 gene expression-implication in p53 mediated apoptosis. Oncogene. 20, 430-439.

- Fujii J., and Taniguchi N. (1991) Phorbol ester induces manganese superoxide dismutase in tumor necrosis factor-resistant cells. J. Biol. Chem. 266, 23142-23146.

- Ho Y.S., and Crapo J.D. (1988) Isolation and characterization of complementary DNAs encoding human manganese-containing superoxide dismutase. FEBS Lett. 229, 256-260.

- Kim H-P., Roe J.H., Chokck, P.B., and Yim M.B. (1999) Transcription activation of the human manganese superoxide dismutase gene mediated by tetradecanoylphorbol acetate. J. Biol. Chem. 274, 37455-37460.

- Kiningham K.K., and St. Clair D.K. (1997) Overexpression of manganese superoxide dismutase selectively modulates the activity of jun-associated transcription factors in fibrosarcoma cells. Cancer Res. 57, 5265-5271.

- Li Y., Huang T.T., Carlson E.J., Melov S.,Ursell P.C., Olson J.L., Noble L.J., Yoshimura M.P., Berger C., Chan P.H., Wallace D.C., and Epstein C.J. (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nature Genetics. 11, 376-381.

- Porntadavity S., Xu Y., Kiningham K.K., Rangnekar M.V., Prachayasitikul V., and St. Clair D.K. (2001) TPA-activated transcription of the human MnSOD gene: Role of transcription factors SP-1 and Egr-1. DNA & Cell Biol. 20, 473-81.

- Safford S.E., Oberley T.D., Urano M., and St. Clair D.K. (1994) Overexpression of manganese superoxide dismutase suppresses metastasis of fibrosarcoma. Cancer Res. 54, 4261-4265.

- St. Clair D.K., Wan X.S., Oberley T.D., Muse K.E., and St. Clair W.H. (1992) Suppression of radiation-induced neoplastic transformation by overexpression of mitochondrial superoxide dismutase. Mol. Carcinogenesis 6, 238-242.

- St. Clair D.K., Oberley T.D., Muse K.E., and St. Clair W.H. (1994) Expression of manganese superoxide dismutase promotes cellular differentiation. Free Radic. Biol. Med. 18, 275-282.

- Visner G.A., Dougall W.C., Wilson J.M., Burr I.A., and Nick H.S. (1990) Regulation of manganese superoxide dismutase by lipopolysaccharide, interleukin-1, and tumor necrosis factor. Role in the acute inflammatory response. J. Biol. Chem. 265, 2856-2864.

- Wan X.S., Devalaraja M.N., and St Clair D.K. (1994) Molecular structure and organization of the manganese superoxide dismutase gene. DNA & Cell Biol. 13, 1127-1136.

- Wispe J.R., Clark J., Burhans M.S., Kropp K.E., Korfhagen T.R., and Whitsett J.A. (1989) Synthesis and processing of the precursor for human mangano- superoxide dismutase. Biochim. Biophys. Acta 994, 30-35.

- Wong G.H W., and Goddel D.V. (1988) Induction of manganese superoxide dismutase by tumor necrosis factor: Possible protective mechanism. Science. 242, 941-944.

- Wong G.H.W., Elwell J.H., Oberley L.W., and Goddel D.V. (1989) Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. Cell. 58, 923-931.

- Xu Y., Kiningham K.K., Devalaraja M.N., Yeh C-C., Majima H., Kasarskis E.J., and St. Clair D.K. (1999) An intronic NF-κB element is essential for induction of the human manganese superoxide dismutase gene by tumor necrosis factor alpha and interleukin-1β. DNA & Cell Biol. 18, 709-722.

-Xu Y., Krisnan A., Wan X.S., Majima H., Yeh C-C., Ludewig G., Kasarskis E.J., and St. Clair D.K. (1999) Mutations in the promoter reveals a cause for the reduced expression of the human maganese superoxide dismutase gene in cancer cells. Oncogene. 18, 93-102.

- Xu Y., Porntadavity S., and St. Clair D.K. (2002) Transcription regulation of the human manganese superoxide dismutase gene: role of specificity protein 1 (Sp1) and activating protein-2 (AP-2). Biochem. J. 362, 401-412.

- Yeh C-C., Wan X.S., and St. Clair D.K. (1998) Transcription regulation of the 5' proximal promoter of the human manganese superoxide dismutase gene. DNA & Cell Biol. 17, 921-930.

- Zhao Y., Oberley T.D., Chaiswing L., Lin S.M., Epstein C.J., Huang T.T., and St Clair D.K. (2002) Manganese superoxide dismutase deficiency enhances cell turnover via tumor promoter-induced alterations in AP-1 and p53-mediated pathways in a skin cancer model. Oncogene. 21, 3836-3846.

- Zhu C-H., Huang Y., Oberley L.W., and Domann E.F. (2001) A family of AP-2 proteins down-regulate manganese superoxide dismutase expression. J. Biol. Chem. 276, 14407-14413.